



Production of monosaccharides and bio-active compounds derived from marine polysaccharides using subcritical water hydrolysis



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ABSTRACT

Polysaccharides are the major components of brown seaweed, accounting for approximately 40–65% of the total mass. The majority of the brown seaweed polysaccharides consists of alginate (40% of dry matter), a linear hetero-polysaccharides commonly developed in fields. However, depolymerisation of alginate is required to recover high-value compounds. In this report, depolymerisation was performed using subcritical water hydrolysis (SWH) at 180–260 °C, with a ratio of material to water of 1:25 (w/v) and 1% formic acid as a catalyst. Sugar recovery was higher at low temperatures in the presence of catalyst. The antioxidant properties of *Saccharina japonica* showed the best activity at 180 °C in the presence of a catalyst. The mass spectra produced using MALDI-TOF showed that polysaccharides and oligosaccharides were produced during hydrothermal treatment. Hydrolysis treatment at 180 °C in the presence of a catalyst may be useful for modifying the structure of *S. japonica* and purified alginate.

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1. Introduction

Seaweed (also known as marine macroalgae) is a heterogeneous assemblage of organisms with a long fossil history, contributing to 85% of the total global production of aquatic plants. Seaweed can be classified into three groups based on pigmentation: green seaweed (Chlorophyta), red seaweed (Rhodophyta), and brown seaweed (Phaeophyta). Seaweeds, multicellular algae rich in minerals and vitamins, are found in marine waters. Seaweed is an important component of food, feed, and medicine, and is considered a high-value marine plant because of its biotechnological properties. The seaweed species most commonly used for its bioactive compounds is brown seaweed. The major constituents of brown seaweed include carbohydrates (45–55%), proteins (10–20%) and lipids (1–10%) (Kumar, Ganesan, Suresh, & Bhaskar, 2008).

Brown seaweed (Phaeophyta) is one of the most abundant seaweed groups of economic importance. The major constituent of brown seaweed is polysaccharide. Alginate or alginic acid is an unbranched hetero-polysaccharide consisting of two hexuronic

acids, β -D-mannuronic acid (ManA or M) and α -L-guluronic acid (GulA or G), which are linked by 1–4 bonds. The physical, chemical, and structural properties of alginic acid (e.g., viscosity, solubility, interaction with metal cations) are directly attributable to the molecular weight and guluronic/mannuronic (G/M) ratio, both of which show important variations among species (Clementi, Crudele, Parente, Mancini, & Moresi, 1999). The biochemical and biophysical properties of alginate are also dependent on the molecular weight and G/M ratio. Alginate has been used in the food industry as gelatinisers and thickeners, as wound coatings and antifouling agents, for gastric parietal protection in various industries (e.g., food and pharmaceuticals), and for the biosorption of heavy metals (Lodeiro et al., 2005; Renn, 1997). Alginate has a molecular weight between 32 and 200 kDa, and the G/M ratio varies by species, age, portion of the plant, and distance from the shore. For applications using alginate, depolymerisation to adjust the molecular weight is required.

The decomposition of carbohydrate into a reducing sugar, monosaccharides, and polysaccharides offers economic value. Many studies have been performed to convert carbohydrates from marine macroalgae into monosaccharides, polysaccharides, and other compounds. Alnaief, Alzaitoun, García-González, and Smirnova (2011) proposed using alginate as a nanoporous biodegradable material, and Fitton, Irhimeh, and Falk (2007) produced marine cosmetics using fucoidan fractions and phloroglucinol. Glucose, mannose, and galactose in brown seaweed belong to the

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reducing sugar group, which contain essential compounds that can be converted into valuable intermediate products.

Alginate depolymerisation methods have been widely investigated. Several methods have been used to adjust the molecular weight and G/M ratio, including acid hydrolysis (Haug, Myklestad, Larsen, & Smidsrod, 1967), base hydrolysis (Rigouin, Delbarre, Siquin, Collic-Jouault, & Dion, 2009), enzymatic depolymerisation (Kim et al., 2012), photolytic depolymerisation (Burana-Osot et al., 2009), and hydrothermal conditions (Aida, Yamagata, Watanabe, & Smith, 2010). Traditional methods use organic solvents such as ethanol, methanol, or hexane during the extraction process (Demirel, Yilmaz-Koz, Karabay-Yavagoslu, Ozdemir, & Sukatar, 2009; Osman, Abushady, & Elshobary, 2010). Although these methods are useful, they have several disadvantages, including the use of harsh chemicals, the need for stable environmental conditions during enzymatic depolymerisation, long reaction times, and high experimental costs. To address these disadvantages, hydrothermal conditions with subcritical water are being used to break down the complex polysaccharide alginate in brown seaweed. Subcritical water is liquid water under pressure at temperatures between the usual boiling point (100 °C) and the critical temperature (374 °C), also known as superheated water and pressurised hot water.

2. Materials and methods

2.1. Materials

The brown seaweed *Saccharina japonica* was collected from Guemil-eup, Wando-gun, Jeollanam-do, South Korea. Purified alginate from brown algae was provided by Sigma Aldrich (United Kingdom). High-purity nitrogen gas (99.99%) was supplied by KOSEM (Yongsan, Republic of Korea). Standards of 2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, catechin, D-glucose, and L-mannose were purchased from Sigma Aldrich Chemical Co. (St. Louis, Mo. USA). Distilled water was used in these experiments. All reagents used in this study were of analytical or high performance liquid chromatography (HPLC) grade and obtained from Sigma Aldrich Chemical Co.

2.2. Sample preparation

After washing fresh *S. japonica* samples with fresh water, unused materials, attached salt, and minerals were removed, and the samples were cut into small pieces. The pieces were dried at –80 °C for 3 days in a freeze dryer (Eyela FDU-2100, Tokyo Rikakikai Co., LTD, Japan) equipped with a square-type drying chamber (Eyela DRC-1000, Tokyo Rikakikai Co., LTD, Japan). The dried samples were collected into sealed plastic bags. The samples were then finely ground using a mechanical blender (PN SMKA-4000 mixer) and sieved through a 710-µm stainless steel sieving mesh.

2.3. Subcritical water hydrolysis

Subcritical water hydrolysis was performed in a 200-cm³ batch reactor made of 276 Hastelloy with temperature control (Meillisa, Chun, & Woo, 2012). A total of 6 g of material samples were loaded into the reactor. Formic acid (1%), which is used as a catalyst, was suspended separately in 150 ml of distilled water. The reactor was then closed and heated using an electric heater to the required temperature (180–260 °C). Pressures were estimated based on saturated steam to be between 15 and 65 bar for the temperature range studied. The temperature and pressure in the reactor were controlled using a temperature controller and pressure gauge, respectively. The sample was stirred using a four-blade stirrer at

140 rpm. The time to reach the desired temperature was 30–75 min. The hydrolysate samples from the reactor were collected and filtered using Whatman nylon membrane filter (0.45 µm) and stored at 4 °C.

2.4. Total glucose

Total glucose measurements were performed using the phenol sulphuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) with minor modifications. Briefly, hydrolysate samples (0.75 ml) were mixed with 2.25 ml of concentrated sulphuric acid. Then, 0.45 ml of 40% phenol was added and the mixture heated in a water bath. The mixture was cooled at room temperature and its absorbance at 490 nm measured using a spectrophotometer (Shimadzu 1240 UV-Vis spectrophotometer). D-Glucose was used as a standard to generate a calibration curve. Each hydrolysate was analysed in triplicate, and the results are expressed in milligrams per litre (mg/l).

2.5. Reducing sugar

Reducing sugar analysis was conducted using the 3,5-dinitrosalicylic (DNS) acid method (Miller, 1959). The reagent solution was prepared by mixing 10 g of sodium hydroxide and 700 ml of water until the mixture completely dissolved. Then, 300 g of potassium sodium tartrate was added to the mixture followed by 10 g of 3,5-dinitrosalicylic (DNS) acid. After all of the components were fully dissolved, 0.5 g sodium sulfite was added followed by 2 g of phenol. The volume of the mixture was adjusted to 1 L and protected from light. Reducing sugar analysis was performed by mixing 0.5 ml of hydrolysate water and 0.5 ml of the reagent solution, heating the mixture for 10 min, and immediately adding 5 ml of cold water. Finally, the absorbance at 540 nm was measured.

2.6. HPLC analysis

Gulose and mannose were quantified using high performance liquid chromatography (HPLC) with an evaporative light scattering detector (ELSD). HPLC analysis was performed using a Jasco HPLC (Easton, USA) model 400 equipped with ChromNav analysis software. High-purity nitrogen (99.99%) from KOSEM Co. was used as a carrier gas. A Shodex (Japan) SUGAR column (SP0810) of 300 mm with 8 mm i.d., thermostated to 80 °C, was used to analyse gulose and mannose compounds. Hydrolysate samples were diluted four-fold using filtered and sonicated water (HPLC grade). The water used for elution was filtered using a Whatman nylon membrane filter (0.45 µm) and sonicated. The flow rate of the eluent was maintained at 0.6 ml/min. Gulose and mannose standards (purity > 98%) were purchased from Sigma Aldrich (United Kingdom).

2.7. Matrix assisted laser desorption/ionisation-time of flight (MALDI-TOF) analysis

The mass spectra of the hydrolysate solutions were obtained using the MALDI-TOF Bruker Autoflex-III, as described by Aida et al. (2010) with minor modifications. Briefly, the device was equipped with a smart beam laser and the spectra were collected with an accelerating voltage of ±19.8 kV, according to the polarity of the recording mode. The measurement was performed using 2,5 dihydroxybenzoic acid (DHBA) as a matrix and acetonitrile:water (75:25) as a solvent. Samples were prepared by mixing 0.5 µl of the product and 0.5 µl of the matrix solution. The matrix solution was prepared by mixing a norharmane-acetonitrile solution and trifluoroacetic acid at a ratio of 7:3. The norharmane-acetonitrile solution was prepared by dissolving norharmane (10 mg) in acetonitrile (1 ml).

2.8. DPPH radical scavenging activity

DPPH assays were performed as described by Sharma and Bhat (2009) with minor modifications. Briefly, 100 µl of sample was mixed with 3.9 ml of methanolic DPPH (50 µM). The mixture was vortexed for 1 min, wrapped in aluminium foil, and incubated at 30 °C for 30 min in darkness. The absorbance of the negative control was measured by replacing the sample with methanol (100 µl). Spectrophotometric measurements of hydrolysate water and the negative control were performed at 517 nm (UVmini 1240, Shimadzu Corp., Japan). The DPPH radical scavenging activity (%) was calculated using the following equation: scavenging activity = $[1 - (As/Ac)] \times 100\%$, where As is the absorbance of the crude extract at 517 nm and Ac is the absorbance of the blank at 517 nm. Analyses were performed in triplicate, and the DPPH activity was expressed as % activity.

2.9. Ferric reducing assay power (FRAP)

The FRAP assay was performed as described by Chu, Chang, and Hsu (2000) with minor modifications. Briefly, 2.5 ml of 0.1 M potassium phosphate buffer (pH 6.6) was mixed with 2.5 ml of 1% potassium ferricyanide. Then, 1 ml of sample was added and the mixture incubated at 50 °C for 20 min. Next, 2.5 ml of 10% trichloroacetic acid, 2.5 ml of water, and 0.5 ml of 0.1% of iron (III) chloride were added to the mixture. The mixture was then incubated at room temperature for 30 min and its absorbance at 700 nm measured using a UV spectrophotometer (UVmini 1240, Shimadzu Corp.). Gallic acid was used as a standard. Analyses were performed in triplicate, and the FRAP of each condition was described in mg/l.

2.10. Total phenolic content (TPC)

The TPC of hydrolysate water was determined with Folin–Ciocalteu reagent as described by Chew et al. (2011) with minor modifications. Briefly, hydrolysate water was diluted using distilled water. A total of 1 ml of diluted sample was mixed with 1 ml of Folin–Ciocalteu reagent (1:10). The mixture was incubated at room temperature for 4 min. Next, 0.8 ml of 7.5% sodium carbonate anhydrous solution was added and the mixture was incubated in darkness for 2 h, after which the absorbance at 765 nm was measured. Gallic acid dissolved in ethanol was used as a standard. Each hydrolysate was analysed in triplicate, and the results were expressed in milligrams per litre (mg/l).

2.11. Total flavonoid content (TFC)

The TFC of hydrolysate water was determined as described by Ozsoy, Can, Yanardag, and Akev (2008) with minor modifications. Briefly, 0.375 ml of hydrolysate water was mixed with 1.88 ml of

water, followed by 112 µl of 5% sodium nitrite. The mixture was allowed to stand for 6 min and 225 µl of 10% aluminium chloride was then added. A total of 0.75 ml of 1 M sodium hydroxide and 0.41 ml of distilled water were added, and the absorbance at 510 nm was measured. Catechin was used as a standard. Each hydrolysate was analysed in triplicate and the results were expressed in milligrams per litre (mg/l).

2.12. pH measurement

The pH of the hydrolysate water was measured using a Mettler Toledo Five Easy Plus pH metre at 20 °C. Prior to the measurements, the pH metre was calibrated using technical buffer solutions of pH 4.01 ± 0.02 , 7.00 ± 0.02 , and 9.2 ± 0.02 .

3. Results and discussion

3.1. Total glucose and reducing sugar

The carbohydrate content in seaweed can be converted into a wide range of water-soluble sugars (such as poly-, oligo-, and monosaccharides) by hydrolysis under subcritical water conditions. The subcritical water technique uses hot water and high pressure to maintain water in the liquid state. Under these conditions, the physical characteristics of water change, with reduced solvent viscosity and surface tension and a lower dielectric constant. Table 1 shows the amounts of total glucose that can be obtained as a function of temperature. The highest recovery was achieved at 180 °C and 13 bar in the absence of a catalyst with purified alginate or *S. japonica*. The amounts of glucose obtained from purified alginate were higher (0.58 g/l) than from *S. japonica* (0.43 g/l). Differentiation of total soluble sugars in hydrolysate water is caused by changes in the physical properties of water, which change in the subcritical state (Daneshvar, Salak, Ishii, & Otsuka, 2012). Glucose recovery was monitored at high concentrations with lower temperatures and pressures, and decreased at higher temperatures and pressures. In the subcritical state, poly-, oligo-, and monosaccharides depolymerised into other byproduct compounds. In fact, the addition of a catalyst increased the decomposition rate. However, the addition of acid during hydrolysis produced byproduct compounds, such as hydroxymethylfurfural (HMF), levulinic acid, and furan aldehyde. Meinita et al. (2012) and Omari, Besaw, and Kerton (2012) described that under certain conditions in which monosaccharides were depolymerised into byproduct compounds, the monosaccharide concentrations in hydrolysate decreased and byproduct compounds increased.

The amounts of reducing sugars (Table 1) obtained from purified alginate in the absence of a catalyst were higher than from *S. japonica*, approximately 0.08–0.12 g/l at 180 °C/13 bar to 260 °C/49 bar. At higher temperatures and pressures, the recovery of reducing sugars in hydrolysate water decreased. Purified alginate

Table 1

Total glucose and reducing sugar recovery (g/l) in different hydrolysis conditions.

	Temperature (°C)/ pressure (bar)	Purified alginate (g/l)		<i>S. japonica</i> (g/l)	
		Total glucose	Reducing sugar	Total glucose	Reducing sugar
With catalyst	180/13	0.45 ± 0.01	0.10 ± 0.00	0.34 ± 0.02	0.05 ± 0.00
	200/17	0.35 ± 0.01	0.07 ± 0.00	0.22 ± 0.02	0.04 ± 0.00
	220/25	0.26 ± 0.03	0.05 ± 0.00	0.14 ± 0.01	0.04 ± 0.00
	240/34	0.17 ± 0.00	0.05 ± 0.00	0.09 ± 0.00	0.04 ± 0.00
	260/49	0.11 ± 0.00	0.05 ± 0.00	0.07 ± 0.00	0.04 ± 0.00
Without catalyst	180/13	0.58 ± 0.00	0.12 ± 0.00	0.43 ± 0.02	0.06 ± 0.00
	200/17	0.51 ± 0.03	0.12 ± 0.00	0.38 ± 0.00	0.06 ± 0.00
	220/25	0.46 ± 0.02	0.11 ± 0.00	0.26 ± 0.01	0.05 ± 0.00
	240/34	0.46 ± 0.00	0.10 ± 0.00	0.22 ± 0.00	0.05 ± 0.00
	260/49	0.39 ± 0.00	0.08 ± 0.00	0.21 ± 0.00	0.04 ± 0.00

consists of high-purity alginate; at increased temperatures, alginate as a polysaccharide was converted into simpler chains such as mono-sugars. Because *S. japonica* is composed of complex compounds (oligosaccharides, fat, protein, minerals, and other compounds), its decomposition rate was lower than that of purified alginate. Moreover, the presence of other compounds during subcritical water hydrolysis affected the carbohydrate decomposition rate. Water-soluble sugars are valuable products of fermentation reactions. Therefore, subcritical water treatment could play an important role as a pretreatment for marine bioethanol production.

3.2. Quantification of gulose and mannose by HPLC

The dominant molecule in brown seaweed is carbohydrate, with alginate identified as a major constituent. Alginate is found in all brown seaweeds as a structural component of the cell wall in the form of insoluble mixed salts of mainly calcium, with lesser amounts of magnesium, sodium, and potassium, and is concentrated in the intracellular space (Venugopal, 2009). Alginate contains three kinds of polymer units: mannuronic (M), guluronic (G), and alternates of the M and G units. In addition to mannuronic and guluronic units of seaweed, it also contains monosaccharides, which have known bioactive activities. The recovery of monosaccharides is required to increase the economic value of seaweed. The depolymerisation method using liquefied hydrothermal processes can be used to break down the polysaccharide polymer in purified alginate or *S. japonica*. The results in Table 2 describe the M and G recoveries in hydrolysate water. The recovery of G and M under all hydrolysate conditions was enhanced in the presence of formic acid as a catalyst. Increased amounts of M and G were obtained from purified alginate in the presence of a catalyst; 3.16 and 6.50 g/l, respectively at 180 °C and 13 bar. The highest recovery from *S. japonica* was 3.24 g/l for mannose and 9.07 g/l for gulose. The highest recovery of G and M also occurred at 180 °C and 13 bar. After increasing the temperature and pressure, G and M concentration of purified alginate and *S. japonica* hydrolysate decreased. Therefore, we can say that G and M is not stable at higher temperature and pressure.

Gulose is an aldohexose sugar belonging to the rare sugars. The International Rare Sugar Society (ISRS) defines a rare sugar as a monosaccharide and derivative that occurs rarely in nature. Although it has been found at low frequencies, this rare sugar has been reported to be a bio-valuable compound. Gulose has been reported to be a building block in the synthesis of nucleoside analogues, which are useful as potential anticancer compounds (Gumina, Song, & Chu, 2001; Sugiura et al., 2007), and a starting material for antiviral medications (Jeong et al., 1993; Tang, 2012). The limited availability of rare sugars have led to estimated

costs for R&D efforts and basic research of \$900/g and \$240/g, respectively (Woodyer, Christ, & Dewese, 2010). Mannose is a type of sugar most commonly used as a source for bio-ethanol production (Saeman, 1945).

3.3. Antioxidant properties

The antioxidant properties of hydrolysate water were examined using antioxidant assays, including total phenolic content (TPC), total flavonoid content (TFC), DPPH radical scavenging activity, and ferric reducing antioxidant power (FRAP). TPC and TFC describe the total amount of phenolics and flavonoids, respectively, in hydrolysate water. The DPPH radical scavenging activity measures the ability of a substance to prevent free radical molecule formation. FRAP can be used to determine total antioxidant power.

As shown in Figs. 1 and 2, the antioxidant properties were higher in the presence of a catalyst. The optimal temperature for antioxidant properties was 180 °C. When purified alginate was used as a starting material, the highest total phenolic, flavonoid, FRAP, and DPPH levels were observed in the presence of catalyst, at 4.06 ± 0.023 mg/l, 1.39 ± 0.002 mg/l, 6.30 ± 0.002 mg/l, and 96.92%, respectively. Similarly, the antioxidant properties (TPC, TFC, FRAP, and DPPH) when *S. japonica* was used as a starting material were 9.63 ± 0.013 mg/l, 1.92 ± 0.05 mg/l, 3.11 ± 0.01 mg/l, and 97.17%, respectively.

The antioxidant properties of hydrolysate water are affected by bio-compounds such as phenolics, flavonoids, and minerals. The growth environment, harvest time, and storage conditions also affected the amounts of these compounds (Roh, Salim-Uddin, & Chun, 2008). The addition of formic acid as a catalyst is known to increase the antioxidant properties of hydrolysate water. Subcritical water has a high ion product H^+ and OH^- value which presents both acidic and basic, and can hydrolyse biomass catalysed by both H^+ and OH^- without adding any acid or base catalysts (Matsumura et al., 2006). H^+ might be increased due to add of acid on subcritical water and produced more bioactive compound. Koivikko, Eränen, Lopenen, and Jormalainen (2008) reported that the presence of acid yields more protection from oxidation and damage by antioxidant compounds, and also changes the solubility and density of water.

The majority of antioxidant compounds found in brown seaweed belong to a group of polar compounds. The general principle of solvent extraction is “like dissolves like”, or in other words, solvents only dissolve substances with similar polarities. Hence, those antioxidant compounds dissolved in water as the solvent. The temperatures above 180 °C used in this process decreased the antioxidant properties of the compounds. It is possible that the antioxidant ability of hydrolysate water was not stable at temperatures above 180 °C.

Table 2
The amounts of gulose and mannose (g/l) in different hydrolysis conditions.

	Temperature (°C)/ pressure (bar)	Purified alginate (g/l)		<i>S. japonica</i> (g/l)	
		Mannose	Gulose	Mannose	Gulose
With catalyst	180/13	3.20 ± 0.2	6.50 ± 0.1	3.20 ± 0.1	9.10 ± 0.2
	200/17	2.50 ± 0.1	5.90 ± 0.2	3.20 ± 0.1	7.60 ± 0.1
	220/25	2.60 ± 0.1	5.90 ± 0.0	2.90 ± 0.1	7.50 ± 0.0
	240/34	2.80 ± 0.1	5.40 ± 0.2	2.60 ± 0.2	6.90 ± 0.1
	260/49	2.70 ± 0.1	5.50 ± 0.1	2.00 ± 0.0	6.30 ± 0.1
Without catalyst	180/13	0.20 ± 0.0	2.70 ± 0.1	1.50 ± 0.1	9.80 ± 0.1
	200/17	0.20 ± 0.0	2.10 ± 0.0	1.20 ± 0.1	8.30 ± 0.1
	220/25	0.10 ± 0.0	2.00 ± 0.0	1.10 ± 0.0	9.00 ± 0.2
	240/34	0.05 ± 0.0	2.00 ± 0.0	1.20 ± 0.0	8.50 ± 0.0
	260/49	0.03 ± 0.0	2.20 ± 0.1	1.00 ± 0.0	9.10 ± 0.1

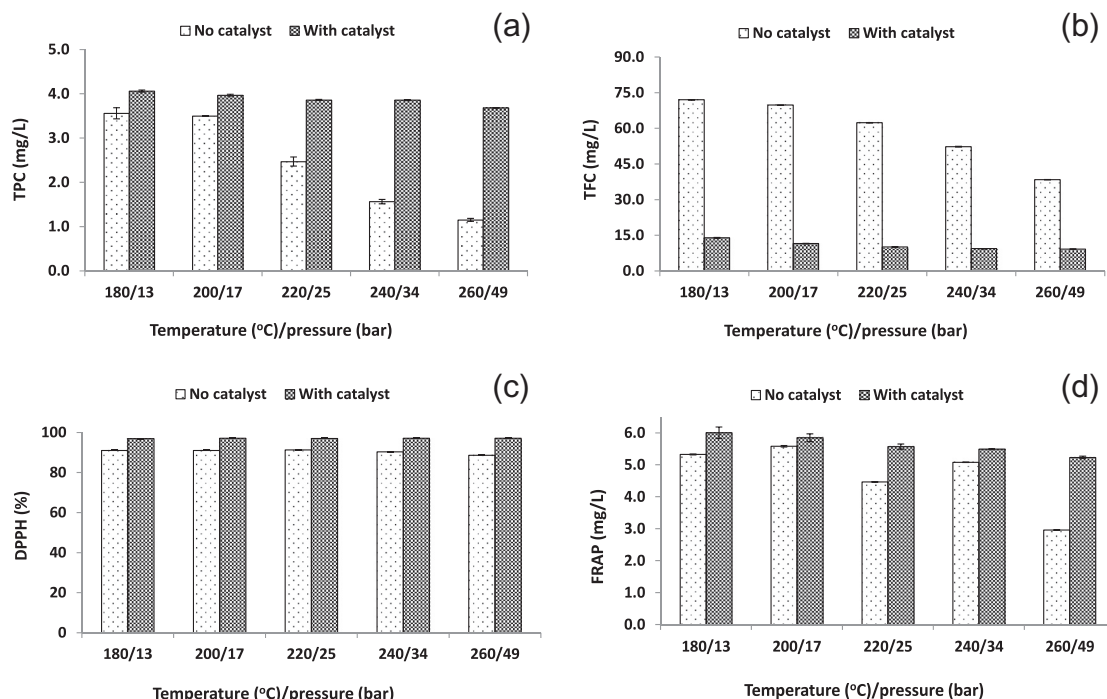


Fig. 1. Antioxidant properties of purified alginate hydrolysate samples under different conditions. Tags: (a) total phenolic content (TPC); (b) total flavonoid content (TFC); (c) DPPH radical scavenging activity; (d) Ferric reducing antioxidant power (FRAP). Data represent the mean values of three replicates \pm S.D.

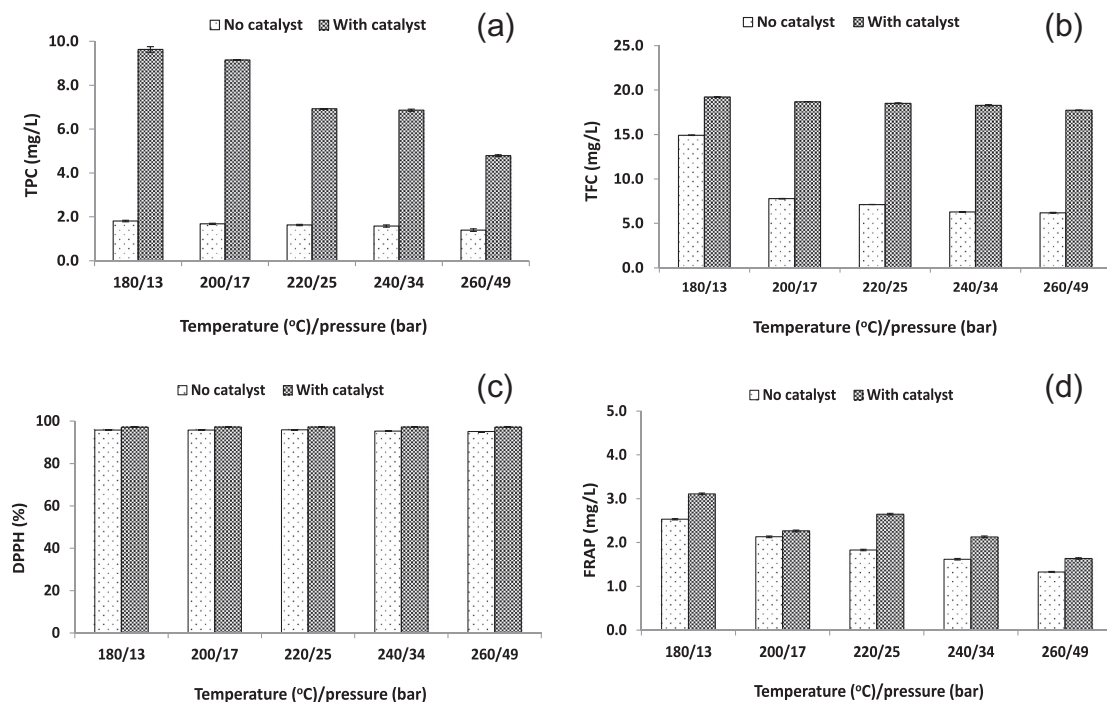


Fig. 2. Antioxidant properties of *S. japonica* hydrolysate samples under different conditions. Tags: (a) total phenolic content (TPC); (b) total flavonoid content (TFC); (c) DPPH radical scavenging activity; (d) Ferric reducing antioxidant power (FRAP). Data represent the mean values of three replicates \pm S.D.

3.4. MW determination of carbohydrate by MALDI TOF mass spectra

MW determination of carbohydrate compounds in *S. japonica* and purified alginate hydrolysate were determined based on MALDI TOF mass spectra. Based on a previous analysis of hydrolysate water, mass spectral analysis was performed during hydrolysis at 180 °C and 13 bar in the presence of catalyst, the

experimental conditions that resulted in high antioxidant activities and sugar recovery. Depolymerisation was accompanied by a reduction in the molecular weight. Fig. 3 shows representative hydrolysate mass spectra from *S. japonica* and purified alginate. Various sizes of MW containing carbohydrate compounds were produced in hydrolysate which were 574, 642, 658, 805, 1221 and 1237 dalton for purified alginate and 574, 658, 674 and

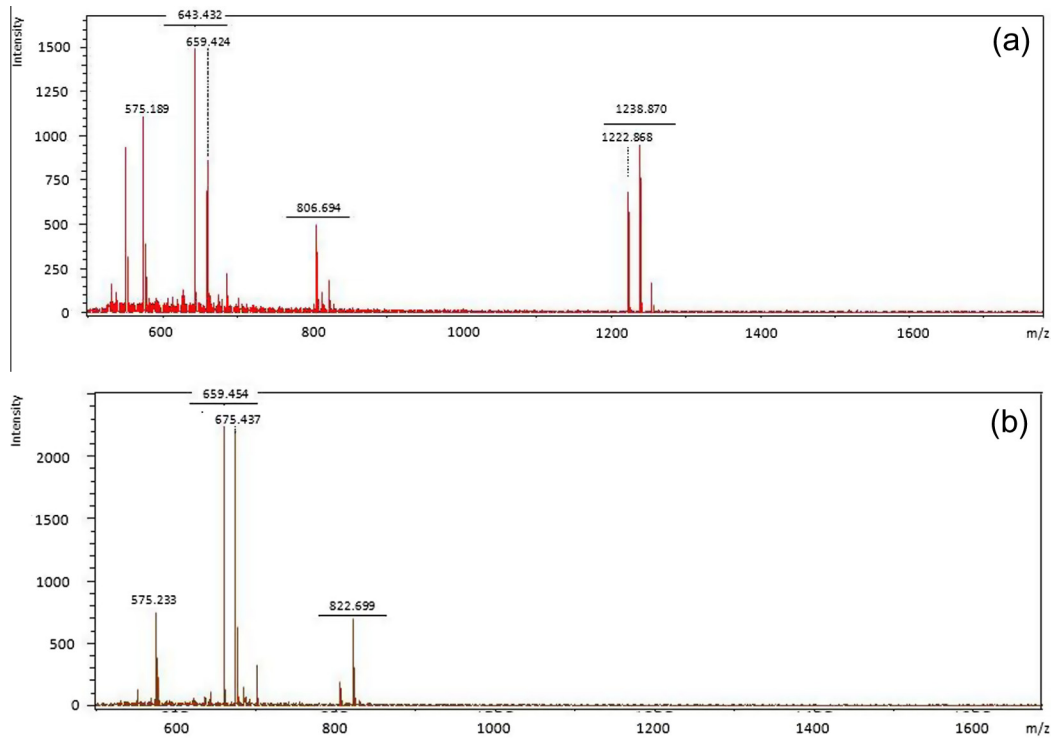


Fig. 3. MALDI-TOF mass spectra of hydrolysate water in the presence of catalyst at 180 °C/13 bar of (a) purified alginate and (b) *S. japonica*.

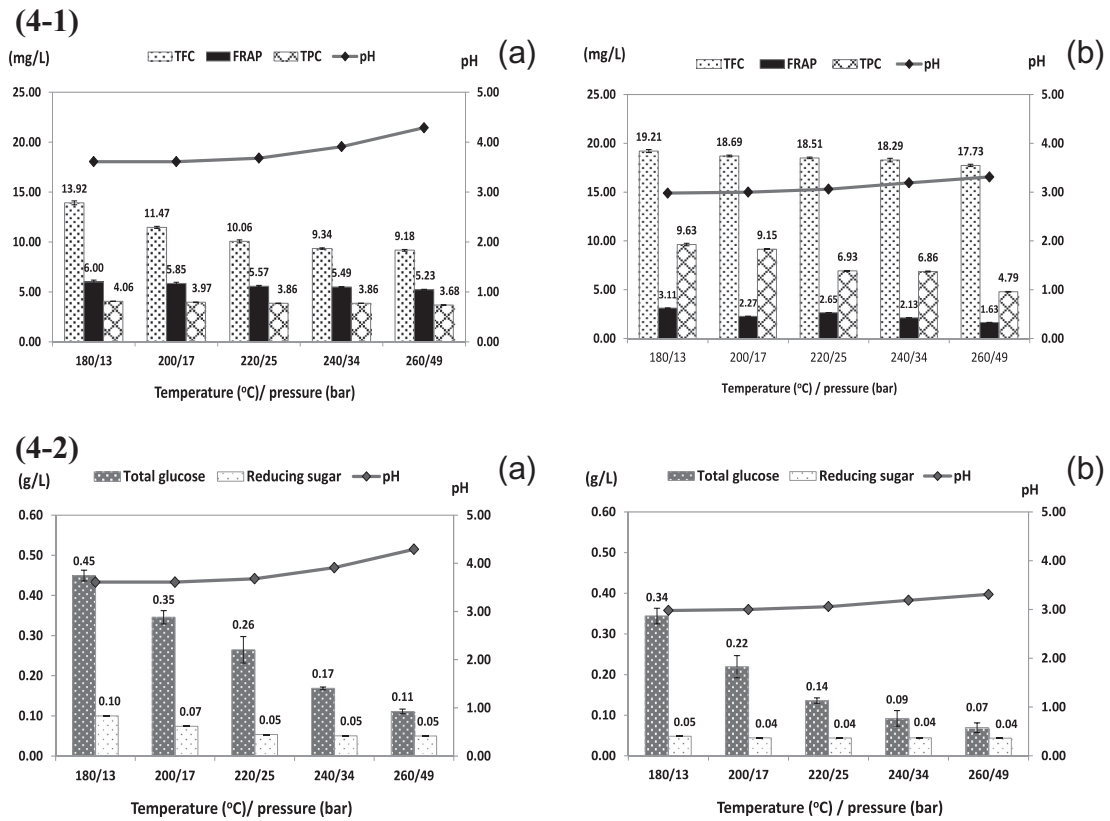


Fig. 4. (4-1) Antioxidant properties and (4-2) sugar recovery of (a) purified alginate and (b) *S. japonica* hydrolysate water affected by pH and temperature.

821 dalton for *S. japonica*. These results show that diversified lower molecular weight carbohydrate compounds were produced during the hydrolysis process.

3.5. Antioxidant properties affect by pH and temperature

Antioxidant properties (TPC, TFC, and FRAP) were examined by measuring the pH in hydrolysate with presence of 1% formic acid at various temperature and pressure shown in Fig. 4–1. The pH values of purified alginate (3.61–4.29) and *S. japonica* (2.98–3.31) hydrolysates were increased at elevated temperatures and pressures. The ions produced in the subcritical state are three orders of magnitude higher than ions in water at ambient conditions and therefore, its potential to act as an acid or base like catalyst. So, the pH values were changed in hydrolysate using various temperature and pressure applied. The antioxidant properties of both purified alginate and *S. japonica* hydrolysates showed an inverse relationship with pH and temperature: when the pH value was greater, produced in hydrolysate at elevated temperature, the antioxidant properties were lower, and *vice versa*. Therefore, lower pH values or higher acidity levels of hydrolysate water provided the best protection against antioxidant compounds. This was also observed by Ragan and Glombitza (1986) and Santos-Buelga and Williamson (2003), and the addition of acid has been used to prevent the oxidation of polyphenolic groups. Pokorny (1986) reported that heating accelerates the initiation reactions and decreases antioxidant activities. The relationship between antioxidant activities and temperature can be understood based on the oxidisability of the antioxidant. Oxidisability is the capability to undergo a chemical reaction with oxygen, namely, the ability to lose hydrogen atoms. The reactivity of antioxidants against free radicals is characterised by the bond dissociation energy (BDE) of the O–H bonds in antioxidant compounds. The easily oxidisable antioxidant compounds showed decreasing antioxidant activities with increasing temperatures. This phenomenon is caused by a decrease in the ability of antioxidants to react with free radicals at higher temperatures. Hence, less oxidisable antioxidants lose their antioxidant activity sooner than easily oxidisable antioxidants (Reblova, 2012).

3.6. Sugar recovery affect by pH and temperature

Sugar recoveries were affected by pH and temperature (Fig. 4–2). At higher temperatures, lower sugar amounts were obtained. Temperature and the chemical interactions between a given component and the water molecule determine the component's solubility in water. In subcritical water states, raising the temperature and pressure significantly change the properties of water. The solvent properties of water vary due to variations in the dielectric constant, conductivity, ionic product, and structure of the H bond network (Galkin & Lunin, 2005).

The solubility of compounds in water is also affected by the saturation cell. The size of the saturation cell varies with the increasing solubility of compounds in water as a function of temperature. A study by King and Srinivas (2012) showed that the solubility of sugar decreases above 413 K and the solubility of flavonoids decreases above 353 K. For the sugar saturation cell, there was evidence of charring inside the saturation cell, which resulted in a dark colour of the collected hydrolysate water. Similar results were reported by Tomasink (1989), showing that dehydration of sugars occurred when heated at temperatures above 373 K. The addition of an acid or base catalyst in subcritical water systems influences the physical properties of water. Espinoza and Morawicki (2012) examined the effect of additives on subcritical water hydrolysis processes. Additives were found to change the physical properties of water, including solubility, polarity, dielectric constant, and viscosity, influencing the solubility of compounds in water.

4. Conclusions

Subcritical water hydrolysis is known to produce monosaccharides and bioactive compounds from marine macroalgae. The antioxidant properties were higher under experimental conditions in the presence of a catalyst, whereas a higher sugar recovery was achieved under experimental conditions lacking a catalyst. In the subcritical state, a chemical reaction occurs between the material and water, changing the pH. The monosaccharides produced during subcritical water hydrolysis process were confirmed as a renewable source of biofuel and bioactive compounds with antioxidant, anticancer, and antiviral properties.

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